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(54) Title: METHODS OF DELIVERING GLP-1

(57) Abstract

Methods of delivering GLP-1 or a GLP-1 mutein, preferably the Gly8 mutein, for the treatment of Type II diabetes and obesity, are disclosed.

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METHODS OF DELIVERING GLP-1

RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to United States
10   Provisional Application 60/081,562, filed April 13, 1998.

FIELD OF THE INVENTION

This invention relates to the treatment of non-insulin dependent diabetes by
delivery of glucagon-like peptide-1-(7-37) (hereinafter GLP-1") and muteins thereof
15   (particularly the Gly$_8$ mutein) at a time and dose sufficient cause therapeutic regulation
of glucose levels within a mammal. Preferably, the GLP-1 is delivered using
encapsulated GLP-1 secreting cells.

BACKGROUND OF THE INVENTION

20   Loss of glucose-induced insulin secretion by pancreatic beta cells is a major
characteristic of non-insulin dependent diabetes mellitus ("type II diabetes"). In the
treatment of type II diabetes, when diet and exercise are no longer able to correct the
diabetic syndrome, postprandial insulin secretion is stimulated by administration of
insulin secretagogues such as, for example, sulfonylureas. These substances stimulate
25   insulin secretion by directly inhibiting the activity of the potassium-ATP channel.
After several years of treatment, however, the initial efficacy of sulfonylureas often
begins to diminish. This has been attributed to beta cell exhaustion resulting from the
action of the sulfonylureas solely on the stimulation of insulin secretion without a
concomitant positive effect on insulin biosynthesis. At present, this limitation to

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treatment with the sulfonylureas has not been overcome and new clinically useful insulin secretagogues are therefore required

One new insulin secretagogue currently being investigated is GLP-1. To date, GLP-1 is perhaps the most potent insulinotropic hormone to be characterized and is

5   widely considered as a potential successful new agent for the treatment of type II diabetes, in part, because its activity is preserved in patients with this disease. One limitation to its therapeutic use, however, is its very short in vivo half-life of approximately five minutes, which is due, in part, to rapid degradation by the endoprotease dipeptidylpeptidase IV.

10   Several studies have been carried out to evaluate the therapeutic potential of GLP-1 in the control of diabetic hyperglycemia in human patients. See, for example, Gutniak, M., Orskov, C., Holst, J.J., Ahren, B., & Efendic, S. Antidiabetogenic effect of glucagon-like peptide 1 (7-36) amide in normal subjects and patients with diabetes mellitus. *N. Engl. J. Med.* 326, 1316-22 (1992). In initial clinical tests, GLP-1 was

15   given intravenously either as a bolus at the beginning of a meal or as continuous infusion over the time of observation. The possible prolonged correction of glycemia after cessation of peptide administration has been recently evaluated.

It has been observed that GLP-1 infused overnight to diabetic patients may lead to correction of basal hyperglycemia, however, discontinuation of GLP-1 infusion just

20   before breakfast was followed by immediate return of glycemia to the diabetic level, indicating no prolonged effect of GLP-1. GLP-1 was also administrated either as subcutaneous injections or as buccal tablets. This led to a good correction of the glycemic excursions but the therapeutic efficacy of the peptide was short-lived.

Habener, United States Patent 5,614,492, incorporated herein by reference,

25   refers to treating type II diabetes with GLP-1. Additionally, several muteins of GLP-1 have been isolated for use in treating type II diabetes. See, e.g. U.S. Patent Nos. 5,118,666; 5,120,712; 5,545,618 (all by Habener) and PCT patent application WO 97/29180, each incorporated herein by reference.

Again, however, the route of administration suggested in those patents (i.e., via

30   i.v. infusion), is done concurrently with the meal because of the short half-life of the peptide *in vivo*. Thus, a deficiency in the art remains.

SUMMARY OF THE INVENTION

This invention provides a method and devices for treating type II diabetes by administering over various time courses a specified continuous dose of a GLP-1 or mutein thereof, to a patient, preferably a GLP-1 mutein having a glycine substitute for

5   alanine at position 8 ("GLP-1-Gly$_8$").

In one embodiment, GLP-1, or preferably a GLP-1 mutein, most preferably GLP-1-Gly$_8$ (having a relatively long half-life) is delivered into the patient at a specified time before a meal. Preferably, the specified time is between 4.0 hours and 0.5 hours before the meal ("pre-prandially"). Most preferably, the GLP-1 mutein is

10   delivered to the patient 0.5 hours pre-prandially. If injected, we contemplate a dosage of GLP-1 or GLP-1 mutein at a rate of about 10-70 µg/day, most preferably 20-50 µg/day (assuming an 80 kg person).

In another embodiment, the GLP-1 is administered by implanting cells or tissue that secrete the GLP-1 or GLP-1 mutein into the patient. In a further embodiment, the

15   GLP-1 secreting cells are encapsulated in at least one device, which is implanted into the patient. While any suitable systemic implantation site is contemplated, we prefer implantion intraperitoneally into the patient.

When delivery is continuous (e.g., using implanted cells or continuous pump, preferably implanted cells, most preferably encapsulated implanted cells), we

20   contemplate providing a continuous dosage of GLP-1 at a rate of about 0.1 - 80 µg/day (assuming a 90 kg person), preferably 0.5- 2.5 µg/day (assuming an 90 kg person).

In another embodiment, when delivery is continuous (e.g., using implanted cells or continuous pump, preferably implanted cells, most preferably encapsulated implanted cells), we contemplate providing a continuous dosage of GLP-1 mutein

25   (preferably the GLP-1-Gly$_8$ mutein) at a rate of about 0.0125 - 2.5 µg/day (assuming a 90 kg person), preferably 0.05- 2.0 µg/day (assuming an 90 kg person).

In another embodiment, when GLP-1 or a GLP-1 mutein is administered continuously, weight gain associated with Type II diabetes is markedly reduced.

BRIEF DESCRIPTION OF THE DRAWINGS

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Fig. 1 shows a strategy for creating a multimeric IgSP-GLP-1-IP2 expression cassette.

Fig. 2 shows various plasmid maps for construction of a multimeric IgSP-GLP-1-IP2 expression cassette.

Fig. 3 shows a list of multimeric GLP-1 expression cassettes.

Fig. 4 shows GLP-1 secretion from BHK cells transfected with a multimeric

GLP-1 expression cassette.

Fig. 5 shows regulation of blood glucose in diabetic and non-diabetic control rats, as well as diabetic rats implanted with GLP-1 secreting encapsulated cell devices.

Fig. 6 shows weight gain over time in diabetic and non-diabetic control rats, as well as diabetic rats implanted with GLP-1 secreting encapsulated cell devices.

DETAILED DESCRIPTION OF THE INVENTION

Glucagon is a 29 amino acid hormone produced in human A-cells of the pancreas. It acts principally to promote glycogenolysis and gluconeogenesis, which causes an increase in the level of blood sugar. Glucagon binds to specific cell-surface receptors found on insulin producing cells. Upon binding glucagon stimulates rapid synthesis of cyclic adenosine monophosphate cAMP, which in turn causes the level of insulin to rise. As the level of insulin rises, it down regulates the production of glucagon, thus completing the feedback loop. In this manner, the expression of glucagon is regulated by insulin which is ultimately regulated by glucose blood levels.

Glucagon is translated from a 630 base pair gene to form the precursor preproglucagon, which is subsequently post-translationally truncated to proglucagon. Proglucagon is subsequently cleaved into three discrete highly homologous individual peptides called glucagon, glucagon-like protein 1, and glucagon-like protein 2. GLP-1 and GLP-2 are 37 and 34 amino acids respectively and are found in the pancreas. By GLP-1 we mean the amino acid sequence of human wild type GLP-1 as presented in United States patents 5,118,666; 5,120,712; 5,545618 and 5,614,492, each incorporated herein by reference. The GLP-1-Gly$_8$ mutein preferred in the present invention has substitution of the alanine normally present at the second position of GLP-1 by a glycine (and otherwise has the amino acid sequence of wild type GLP-1). This substitution confers resistance to the peptide from hydrolysis by the purified dipeptidyalpeptidase IV ("DPPIV") enzyme and preserves biological activity of the mutant peptide incubated for up to 72 hours in the presence of FBS, which naturally contains the DPPIV enzyme compared to the decreased activity of the wild-type peptide

under identical conditions. Importantly, this Ala to Gly substitution did not change the binding affinity nor the capacity of the peptide to activate the production of cAMP. The effectiveness of the DPPIV-resistant form of the mutein peptide to control glycemic levels in glucose intolerant mice was demonstrated the following by several

5   experiments.

First, when injected at 30 minutes after the initiation of an interperitoneal glucose tolerance test ("IPGTT"), GLP-1-Gly$_8$ showed an approximately 10-fold more potent therapeutic effect with 0.1 nanomole of the mutant peptide giving the same glycemic correction as 1 nanomole of GLP-1. This effect was mediated by the

10   insulinotropic effect of the peptide since insulin levels rose and glucagon levels decreased 30 minutes after injection of the mutant peptide.

Second, pre-injection of one nanomole of GLP-1-Gly$_8$ up to 4 hours before initiation of the IPGTT completely corrected the glucose intolerance whereas the same amount of GLP-1 had no effect even when injected 30 minutes before the IPGTT.

15   Third, when injected 2 hours before the IPGTT, 0.3 nanomole completely corrected the glucose intolerance and 0.1 nanomole had a significant effect.

This improved physiological action of GLP-1-Gly$_8$ was unexpected as previously published data reported that the half-life of GLP-1-Gly$_8$ incubated *in vitro* in pig serum was increased by approximately only 6-fold and that its half-life when

20   injected intravenously in pigs was increased by approximately only 3-fold as compared to GLP-1. The basis for the improved biological efficacy of this peptide is so far not known. It may however be due to the fact that cleavage of GLP-1 by DPPIV leads to the formation of GLP-1(9-37). This 9-37 peptide is a low affinity antagonist of the GLP-1 receptor and can be found, *in vitro*, in a 10-fold excess over GLP-1. Because of

25   its resistance to DPPIV, GLP-1-Gly$_8$ is unlikely to give rise to significant concentrations of this antagonist. This may therefore explain, in part, the persistence of the *in vitro* efficacy of the mutated peptide.

In addition, a single injection of GLP-1-Gly$_8$ increased insulin and decreased glucagon plasma levels much more efficiently than GLP-1 and for a prolonged period

30   of time. The improved insulin to glucagon ratio may have significant effects on hepatic gene expression which may lead to long-term control of hepatic glucose production thereby further reinforcing the antidiabetic effect of GLP-1-Gly$_8$.

As a result, GLP-1-Gly$_8$, due to its resistance to inactivation by DPPIV and probable absence of antagonist production, increases insulin secretion and decreases glucagon plasma levels for a longer time than the natural peptide. These combined effects lead to prolonged improvement of glucose homeostasis, possibly through

5   alteration of gene expression, in particular those coding for enzymes involved in hepatic glucose metabolism.

GLP-1-Gly$_8$ therefore represents a significantly improved form of this incretin hormone since lower doses could be used for the correction of diabetic hyperglycemia and with a more flexible schedule of administration.

10   Thus in one embodiment, a schedule of administration and dosages of GLP-1-Gly$_8$ is provided. In this embodiment, the patient is administered preprandially a therapeutic amount of a GLP-1-Gly$_8$ mutein, the mutein having the following formula:

His-Xaa^1-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Xaa^2-Gly-Gln-Ala-
15   Ala-Xaa^3-Xaa^4-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Xaa^5 (SEQ ID NO 1)
        wherein
        Xaa^1 Gly, ;
        Xaa^2 is Glu, Gln, Ala, Thr, Ser, and Gly;
        Xaa^3 is Lys, and Arg;
20   Xaa^4 is Glu, Gln, Ala, Thr, Ser, and Gly; and
        Xaa^5 is Gly-OH or is absent;

In this embodiment, the GLP-1 or GLP-1 mutein is delivered preprandially preferably between 0.5 hours and 4.0 hours. More preferably, the GLP-1 or GLP-1 mutein is delivered preprandially at approximately 0.5 hours. We contemplate a

25   number of modes of delivery, including via injection, orally in a pharmaceutically acceptable formulation (preferably in a buccal tablet), or via continuous delivery using slow release polymers or encapsulated cell-based delivery.

In one preferred cell-based delivery embodiment, a continuous dose of GLP-1 or GLP-1 mutein (most preferably GLP-1-Gly$_8$) is provided by implanting cells or

30   tissue secreting the GLP-1 or GLP-1 mutein (preferably the mutein of SEQ ID NO. 1, most preferably the GLP-1-Gly$_8$ mutein having a Gly at position 8 and otherwise having the same amino acid sequence as wild type GLP-1) into the patient.

The cells can be implanted either unencapsulated or encapsulated in a semipermeable membrane. If unencapsulated cells are implanted, the patient may need

35   to be treated concommittently with immunosupressive drugs. We prefer use of

encapsulated cells or tissue. Accordingly, in a preferred embodiment, GLP-1 secreting

cells or GLP-1 mutein secreting cells (preferably GLP-1-Gly$_8$ secreting cells) are

encapsulated in a semipermeable membrane prior to implantation within the host. The

cells continuously secrete the GLP-1 or GLP-1 mutein which passes through the

5   semipermiable membrane into the patient where it exhibits its therapeutic effect.

Many different cell types may be encapsulated in the device disclosed by the

present invention. These include well known, publicly available immortalized cell lines

as well as dividing primary cell cultures. Examples of suitable cell lines include

Chinese hamster ovary cells (CHO); baby hamster kidney cells (BHK); mouse

10   fibroblast-3T3 cells; African green monkey cell lines (including COS-1, COS-7,

BSC-1, BSC-40, BMT-10 and Vero); rat adrenal pheochromocytoma (PC12 and

PC12A); AT3, rat glial tumor (C6); EGF-responsive neurospheres; bFGF-responsive

neural progenitor stem cells derived from the CNS of mammals [Richards *et al.*, *PNAS*

89: 8591-8595 (1992); Ray *et al.*, *PNAS* 90: 3602-3606 (1993)]; primary fibroblasts;

15   Schwann cells; astrocytes; ß-TC cells,;Hep-G2 cells; oligodendrocytes and their

precursors; mouse myoblast cells-C2C12; human glial-derived cells-Hs683; human

glial-derived cells-A172; porcine glioblasts; chondroblasts isolated from human long

bone; rabbit corneal-derived cells (SIRC), and CAC cells.

In a preferred embodiment, the encapsulated cells are transfected with DNA

20   molecules comprising DNA sequences coding for GLP-1 or a GLP-1 mutein. The

DNA encoding GLP-1 and various GLP-1 muteins, including the GLP-1-Gly$_8$ mutein,

are known.

Methods of using encapsulated cells for the delivery of biologically active

molecules is disclosed in US patent 5,653,975, herein incorporated by reference.

25   Methods for the use of encapsulated cells to deliver biologically active moieties and to

provide metabolic functions (such as removal of harmful substances) are known. See,

e.g., US patent 5,800,828, herein incorporated by reference.

Methods for controlling cell distribution within an encapsulation device have

also been discussed. See, *e.g.*, US patent 5,795,790, herein incorporated by reference.

30   his invention contemplates encapsulating several different cell types. For

example, a patient may be implanted with a capsule device containing a first cell type

(e.g., BHK cells). If after time the patient develops an immune response to that cell

type, the capsule can be retrieved, or explanted, and a second capsule can be implanted

containing a second cell type (e.g., C_2C_{12} cells). In this manner, continuous provision of the therapeutic molecules is possible, even if the patient develops an immune response to one of the encapsulated cell types.

In one preferred embodiment myoblasts, most preferably human myoblasts, are
5   transformed with a DNA encoding GLP-1 or a GLP-1 mutein. Schematic representations of GLP-1-Gly$_8$ expression vectors are provided herein.

Encapsulation hinders elements of the immune system from entering the capsule, thereby protecting the encapsulated cells from immune destruction. The semipermeable nature of the capsule membrane also permits the molecule of interest to
10   easily diffuse from the capsule into the surrounding host tissue. This technique limits the risk of tumor formation inherent in cell-based therapies and allows the use of unmatched human or even animal tissue, without immunosuppression of the recipient. Moreover, the implant may be retrieved if necessary or desired.

As used herein "a biocompatible capsule" means that the capsule, upon
15   implantation in a host mammal, does not elicit a detrimental host response sufficient to result in the rejection of the capsule or to render it inoperable, for example, through degradation.

A variety of biocompatible capsules are suitable or delivery of molecules according to this invention. Such capsules will allow or the passage of metabolites,
20   nutrients and therapeutic substances while minimizing the detrimental effects on the host immune system. Numerous encapsulation devices are known, having various outer surface morphologies and other mechanical and structural characteristics.

Useful biocompatible polymer capsules are comprised of (a) a core which contains a cell or cells, either suspended in a liquid medium or immobilized within an
25   immobilizing matrix; and (b) surrounded or peripheral region of permselective matrix or membrane (jacket) which does not contain isolated cells, which is biocompatible, and which is sufficient to protect isolated cells if present in the core from detrimental immunological attack.

Some transformed cells or cell lines are most advantageously isolated within a
30   capsule having a liquid core. For example, cells can be isolated within a capsule whose core comprises a nutrient medium, optionally containing a liquid source of additional factors to sustain cell viability and function, such as fetal bovine or equine serum.

Alternatively, the core may be composed of an immobilizing matrix which stabilizes the position of the cells. The matrix may be formed from a hydrogels or extracellular matrix molecule (or components thereof), or other suitable material (e.g., non-woven polyester fabrics, foams, or other biocompatible immobilizing material),

5   preferably a biocompatible hydrogel. See, e.g., WO92/19195 and WO95/05452.

The term "hydrogel" herein refers to a three dimensional network of cross-linked hydrophilic polymers. The network is in the form of a gel, substantially composed of water, preferably but not limited to gels being greater than 90% water.

Compositions which form hydrogels fall into three classes. The first class

10   carrier a net negative charge (e.g., alginate). The second class carrier a net positive charge(e.g., collagen and laminin). Examples of commercially available extracellular matrix components include Matrigel™ and Vitrogen™. The third class is net neutral in charge (e.g., highly crosslinked polyethylene oxide, or polyvinylalcohol). Any suitable matrix or spacer may be employed within the core, including precipitated chitosan,

15   synthetic polymers and polymer blends, microcarriers and the like, depending upon the growth characteristics of the cells to be encapsulated.

Various polymers and polymer blends can be used to manufacture the capsule jacket, including, but not limited to, polyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethanes, polystyrenes,

20   polyamides, cellulose acetates, cellulose nitrates, polysulfones (including polyether sulfones), polyphosphazenes, polyacrylonitriles, poly(acrylonitrile/covinyl chloride), as well as derivatives, copolymers and mixtures thereof.

In a preferred configuration, the capsule jacket comprises a biocompatible semipermeable hollow fiber membrane. Such membranes, and methods for making

25   them are disclosed in United States patents 5,284,761 and 5,158,881, herein incorporated by reference. In a preferred embodiment, the capsule jacket is formed from a polyether sulfone hollow fiber, such as those described in United States Patent Nos. 4,976,859 and 4,968,733, herein incorporated by reference. An alternate preferred jacket material is poly(acrylonitrile/covinyl chloride).

30   Preferably, the capsules are immunoisolatory. To be immunoisolatory, the surrounding or peripheral region of the capsule should confer protection of the cells from the immune system of the host in whom the capsule is implanted, by preventing

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harmful substances of the host's body from entering the core of the vehicle, and by providing a physical barrier sufficient to prevent detrimental immunological contact between the isolated cells and the host's immune system. The thickness of this physical barrier can vary, but it will always be sufficiently thick to prevent direct contact

5   between the cells and/or substances on either side of the barrier. The thickness of this region generally ranges between 5 and 200 microns; thicknesses of 10 to 100 microns are preferred, and thickness of 20 to 75 microns are particularly preferred. Types of immunological attach which can be prevented or minimized by the use of the instant vehicle include attack by macrophages, neutrophils, cellular immune responses (e.g.

10   natural killer cells adn antibody-dependent T cell-mediated cytolysis (ADCC), and humoral response (e.g., antibody-dependent, complement-mediated cytolysis).

        An immunoisolatory capsule can be used for the delivery, even from xenogeneic cells, of products having a wide range of molecular sizes. Accordingly, nominal molecular weight cutoff (MWCO) values from 50 kD up to 1000 - 2000 kD are

15   contemplated. Preferably, the MWCO is between 70-300 kD.

        The capsule can be any configuration appropriate for maintaining biological activity and providing access for delivery of the product or function, including for example, cylindrical, rectangular, disk-shaped, patch-shaped, ovoid, stellate, or spherical. Moreover, the capsule can be coiled or wrapped into a mesh-like or nested

20   structure. If the capsule is to be retrieved after it is implanted, configurations which tend to lead to migration of the capsules from the site of implantation, such as spherical capsules small enough to travel in the recipient's blood vessels, are not preferred. Certain shapes, such as rectangles, patches, disks, cylinders, and flat sheets offer greater structural integrity and are preferable where retrieval is desired.

25   In one preferred embodiment, the implantable capsule is of a sufficient size and durability for complete retrieval after implantation, and preferably the device has a tether that aides in retrieval. Such tethers are well known in the art. Such macrocapsules have a core of a preferable minimum volume of about 1 to 10 μl and depending upon use are easily fabricated to have a volume in excess of 100 μl.

30   In a hollow fiber configuration, the fiber will have an inside diameter of less than 1500 microns, preferably between 300-900 microns. In either geometry, the hydraulic permeability will be in the rate of 25 to 70 mls/min/m²/mmHg. The glucose

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mass transfer coefficient of the capsule, defined, measured and calculated as described by Dionne et al., ASAIO Abstracts, p. 99 (1993), and Colton et al., The Kidney, eds., Brenner BM and Rector FC, pp. 2425-89 (1981) will be greater than 10^{-6} cm/sec, preferably greater than 10^{-4} cm/sec.

5   Cell loading density may be varied over a wide range. We contemplate encapsulation of between 10^4 to 10^9, preferably 10^5 to 10^8 cells, in each device. Cells, either in aqueous solution or in a supporting matrix (e.g., a hydrogel, extracellular matrix components or supporting or immobilizing matrix), are then injected into the membrane and the ends of the device are sealed.

10   Any suitable method of sealing the capsules may be used, including the employment of polymer adhesives and/or crimping, knotting and heat sealing. These sealing techniques are known in the art. In addition, any suitable "dry" sealing method can also be used, as described in United States patent 5,738,673, incorporated herein by reference. In such methods, a substantially non-porous fitting is provided through
15   which the cell-containing solution is introduced. Subsequent to filling, the capsule is sealed.

          The methods and devices of this invention are intended for use in a mammalian host, recipient, patient, subject or individual, preferably a primate, most preferably a human. A number of different implantation sites are contemplated for the devices and
20   methods of this invention. These implantation sites include systemic implantation, including subcutaneous (preferable), intraperitoneal (also preferable), intravenous, or intramuscular implantation, and implantation into the central nervous system, including the brain, spinal cord, and aqueous and vitreous humors of the eye, intrathecally, and into the lateral ventricles.

25   When delivery is continuous (e.g., using implanted cells or continuous pump, preferably implanted cells, most preferably encapsulated implanted cells), we contemplate providing a continuous dosage of GLP-1 at a rate of about 0.1 - 80 µg/day (assuming a 90 kg person), preferably 0.5- 2.5 µg/day (assuming an 90 kg person).

          In another embodiment, when delivery is continuous (e.g., using implanted cells
30   or continuous pump, preferably implanted cells, most preferably encapsulated implanted cells), we contemplate providing a continuous dosage of GLP-1 mutein

(preferably the GLP-1-Gly$_8$ mutein) at a rate of about 0.0125 - 2.5 µg/day (assuming a 90 kg person), preferably 0.05- 2.0 µg/day (assuming an 90 kg person).

The actual dosage can be varied by any suitable method known in the art, including, e.g., by implanting a fewer or greater number of capsules. For

5   macrocapsular delivery, we prefer between one and ten capsules.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. These examples should in no way be construed as limiting the scope of the invention, as defined by the appended claims.

EXAMPLES

10   **Example 1: Resistance of GLP-1-Gly8 to Dipeptidylpeptidase IV**

GLP-1, GLP-1-Gly$_8$ and diprotin A were purchased from Bachem, Switzerland, and were HPLC purified to 97%. Peptides were incubated in the presence of 20µM of purified DPPIV in a total volume of 50µl of 25 mM triethanolamine pH 7.8 for 15 min at 37°C. The incubation reaction was then acidified and peptides analyzed by HPLC

15   using a Nucleosil phenyl reverse-phase column (125 x 3.0 mm, Macherey-Nagel, Oensingen, Switzerland) using isocratic elution conditions. The sensitivity of the method allowed the detection of 0.1% of degradation product GLP-1(9-37). Intact GLP-1 and GLP-1-Gly$_8$ eluted at a retention time of approximately 7.5 minutes. The GLP-1-(9-37) eluted at a retention time of approximately 10.5 minutes. In conditions

20   where as much as 10 % of GLP-1 was converted to GLP-1-(9-39), less than 0.1 % GLP-1-Gly$_8$ was converted to the truncated from, thus indicating marked resistance of GLP-1-Gly$_8$ to DPPIV.

Alternatively, peptides were incubated, at an initial concentration of 10nM, in DMEM containing 10% FBS, which naturally contains DPPIV, at 37°C for different

25   periods of time and in the presence or absence of 0.2 mM diprotin A, which is an inhibitor of DPPIV. The stability of GLP-1-Gly$_8$ was analyzed by assaying the bioactivity of the remaining peptide by measuring its ability to activate cAMP production in Chinese hamster lung fibroblasts that stably express the rat GLP-1 receptor (clone 5 cells). Production of intracellular cAMP in clone 5 cells was induced

30   by adding an aliquot of the incubation medium diluted 1:10 to the cell culture. The activity of the sample was compared to a standard dose-response curve for cAMP

production generated using the corresponding peptide diluted in the same incubation medium.

The half-life of GLP-1 was about 35 hours when incubated in the presence of FBS and was extended to 65 hours when diprotin A was present. In contrast, under the 5 same conditions, GLP-1-Gly$_8$ remained stable for more than 72 hours and was not affected by the presence of diprotin A.

Example 2: Binding of GLP-1-Gly8 to the GLP-1 Receptor

Binding of GLP-1-Gly8 to the GLP-1 receptor was evaluated in displacement 10 experiments. Chinese hamster lung fibroblasts expressing GLP-1 receptor (Clone 5) cells were incubated in the presence of tracer amounts of radioiodinated GLP-1. Increasing concentrations of wild-type or mutant peptides were added to displace the radiolabeled material. The amount of radioactivity in the cell supernatant was then quantified and plotted vs. peptide concentration. The IC 50s for displacement of bound 15 radioiodinated GLP-1 by GLP-1 or GLP-1-Gly$_8$ were not statistically different (0.41 ±- 0.14 nM vs. 1.39 ±- 0.61 nM, respectively). The data are the mean ± SE of 4 different experiments. Dose dependent induction of cAMP accumulation in clone 5 cells by both peptides also displayed the same EC50s. Again, the data are the mean ± SE of 4 different experiments. The EC50 (nM, mean ± SE) is indicated for both peptides.

20

Example 3: In vivo studies of GLP-1 and GLP-1-Gly8 in mice

Six week old male C57B1/6JIco mice weighing 18 to 20 g were obtained from IFFA Credo, L'Arbresle, France. The mice were fed ad libitum either with normal chow pellets ("NC") or a high fat diet ("HF"). The NC chow had an energy content of 25 12 % from fat, 28 % from protein, and 60 % from carbohydrates. The HF chow had an energy content of 72 % from fat, 28 % from protein and less that 1 % from carbohydrate. After eight weeks of diet the HF fed mice had a body weight of 30.2 ±- 0.6 grams and the NC fed mice had a body 28.1 ±- 0.3 grams. Over the course of the study, the HF fed mice weighed approximately 30 % more than the NC fed mice, with 30 average body weights of 38.5 ±- 0.8 g to 30.3 ±- 3 g for the HF and NC mice, respectively.

13

The presence of a diabetic phenotype was determined by measuring blood glucose, glucagon, and insulin for fed mice and fasted mice at various time intervals. Table 1, below, shows that after feeding, blood glucose levels were lower in HF mice than in the NC mice as a result of the absence of carbohydrates from the HF diet.

5   Fasting, however, induced a rapid decrease in blood glucose concentrations in the NC mice whereas in the HF group glycemia remained relatively constant (5.7 ±- 0.2 mM and 7.2 ±- 0.1 mM after six hours fast in NC and HF mice, respectively).

Table 1 shows the results after 24 hours. Insulin levels in the fed state were lower in the HF mice as compared to the NC mice as shown in Table 1. Twenty four

10   hours of fasting decreased insulin concentrations in both groups and insulin levels were significantly lower in the HF group compared to the NC group. Table 1 also shows that glucagon levels in the fed state were higher in the HF group than in the NC mice, but were no different after fasting. Taken together, these data indicate that the high fat feeding induced a diabetic phenotype on the HF mice.

15

Table 1: Glucose, insulin, and glucagon levels in fed and 24 hours Fasted C57B1/6J mice after 16-18 weeks of NC or HF diet

	Fed		Fasted	
	NC	FC	NC	HF
Glucose (Mm)	8.2±0.2*	43.8±8.4	4.9±0.2*	6.9±0.4
Insulin (μU/ml)	76.3±7.8*	43.8±8.4	19.8±4.9*	9.7±1.4
Glucagon (pg/ml)	59.2±2.0*	117.7±7.4	35.5±3.2	28.8±5.5

* significantly different from the corresponding HF group. P<0.05, n≤5.

20

GLP-1 and GLP-1-Gly8 were also evaluated for their ability to correct the glycemic excursions of HF mice during intraperitoneal glucose tolerance tests (IPGTT) performed after a five hour period of fasting. To determine blood glucose levels, 2 μl of blood was removed from the tail vein of the mice and glucose level was determined

25   using a glucose meter (Bayer, Zurich, Switzerland). The results were plotted for various time points and the area under the curve was determined ("AUC"). Insulin and

glucagon were analyzed by radioimmunoassays (Linco, St Louis, MO) from blood collected from the orbital sinus of separate sets of animals.

Figure 1 shows the IPGTT curves performed with NC and HF mice. The HF mice show a higher glycemia at the time of glucose injection and a much higher and
5   prolonged increase in blood glucose. This is reflected by the 1.7-fold higher area under the curve for the first two hours. (AUC(0-120) for HF compared to NC mice. Table 2, shown below, presents the blood glucose, plasma insulin and glucagon levels 30 minutes after the initiation of the IPGTT. Glycemia was two-fold higher and plasma insulin was 50% lower in HF compared to control mice and glucagon levels were the
10   same (Table 2). There was thus impaired insulin to glucose and insulin and insulin to glucagon ratios characteristic of type II diabetes. When injected at 30 minutes, one nanomole of GLP-1 led to a correction of the AUC calculated between 60 and 120 minutes. This correction was however not as good as with one nanomole of GLP-1-Gly8. But similar to that obtained with 0.1 nanomole, of GLP-1-Gly8. Furthermore,
15   with the mutant, but not with the wild-type peptide, the correction of glycemia was associated with a rise in circulating insulin and a decrease in plasma glucagon measured at 60 minutes after initiation of the IPGTT as shown in Table 2.

Table 2: Plasma hormones and glucose levels in HF fed mice during an IPGTT.

An IPGTT (1 g/kg body weight) was performed with 5 hours fasted NC or HF mice. 30 minutes after initiation of the test, the HF mice received an i.p. injection of saline, or of saline containing 1 nanomole of GLP-1 or GLP-1-Gly8. Glucose, insulin, and glucagon levels were measured at 60 minutes.

| | Mice at 30 min of IPGTT | | HF mice at 60 min of IPGTT after injection at 30 min. of: | | |
	NC	HF	Saline	GLP-1	GLP-1-Gly8
Glucose (Mm)	10.4±0.7*	20.2±1.5	13.5±0.2	11.1±0.7[a]	7.2±1.7[a]
Insulin (μU/ml)	55.2±6.1*	24.6±4.9	8.0±0.9	6.8±1.0	25.7±1.2[a]
Glucagon (pg/ml)	59.1±2.0	53.2±4.0	53.0±3.7	51.1±11.1	38.2±2.9[a]
Insulin Glucose ratio	5.5	1.2	0.51	0.75	2.44[a]
Insulin Glucagon ratio	0.93	0.49	0.15	0.12	0.67[a]

* : significantly different from the corresponding HF group, $p < 0.05$, $n \leq 5$.

a : Significantly different from the corresponding HF saline group, $p < 0.05$, $n \leq 5$.

GLP-1 or GLP-1-Gly8 were then injected into the peritoneal cavity of HF mice at several time points before the initiation of an IPGTT. When preinjected at -30 minutes one nanomole of GLP-1 did not normalize the glucose tolerance whereas one nanomole of GLP-1-Gly8 completely normalized it. This was quantified by calculating the AUC(0-120) of the glucose tolerance test. The glucose AUC(0-120) was also corrected when one nanomole GLP-1-Gly$_8$ was injected intraperitoneally up to 4 hours, but not 24 hours, before the start of the IPGTT. When administered intraperitoneally 2

hours before initiation of the IPGTT, complete correction of the glucose intolerance could be achieved with 0.3 nanomole of GLP-1-Gly8 and that a significant effect was still observed with 0.1 nanomole.

The ability of GLP-1-Gly8 to control glycemia in 5 hours fasted mice was then

5   established. A single injection of one nanomole of GLP-1-Gly8 could maintain normoglycemia for at least 4 hours whereas the effect of one nanomole of GLP-1 had already vanished after 3 minutes. Alternatively, when GLP-1-Gly8 was injected 2 hours before blood was sampled, normoglycemia was maintained with as low as 0.1 nanomole as shown in Table 3.

Table 3: Effect of GLP1 on fasting blood glucose levels.
C57B1/6J mice fed a NC or a HF diet were fasted for 5 hours before the blood glucose
measurements were performed. The mice had previously received 1 nanomole of GLP-
1 or GLP1-Gly8 as i.p. injection at the indicated times before blood sampling or the
indicated amount of GLP-1-Gly8 2 hours before the measurement of glycemia.

Groups	Treatment	Time of Injection	Fasted Blood Glucose levels mM
NC	No	-	5.7 ± 0.2*
HF	No	-	7.2 ± 0.1
	GLP-1: 1 nmole	-10 min - 30 min	6.0 ± 0.1* 7.8 ± 0.5
	GLP-1-Gly8: 1 nmole	-10 min -30 min -4 hrs -24 hrs	5.3 ± 0.1* 5.9 ± 0.4* 6.1 ± 0.3* 7.4 ± 0.3
NC	Saline	-2 hours	5.4 ± 0.2[a]
HF	Saline GLP-1-Gly8 1.0 nmole 0.3 nmole 0.1 nmole 0.03 nmole	-2 hours -2 hours	6.9 ± 0.8 5.5 ± 0.3[a] 5.7 ± 0.2[a] 5.5 ± 0.3[a] 6.6 ± 0.3

* = significantly different from the corresponding not injected HF group, $p < 0.05$, $n \leq 5$.

a = significantly different from the corresponding saline injected HF group, $p < 0.05$, $n \leq 5$.

Example 4 **GLP-1-Gly8 expression plasmid**

Materials

All restriction, modifying, and Taq polymerase enzymes were purchased from
Life-Technologies (Basel, Switzerland). The TOPO-TA Cloning kits were obtained
from Invitrogen Corporation (Carlsbad, CA., U.S.A.).

Oligonucleotides

Oligonucleotides were custom synthesized by Life-Technologies (Basel,

Switzerland) and/or Microsynth (Balgach, Switzerland). Oligonucleotides synthesized

5   for the cloning of the multimeric IgSP-GLP1-IP2 expression cassettes included:

oMODEX-1025: 5'-CCCGCTAGCGCGTCACCCCTAGAGTCGAGCTGTG-3';

oMODEX-1026: 5'- TCCCTTCACCATGCGAATTCACCCCTGTAG-3';

oMODEX-1027: 5'- ACAGGGGTGAATTCGCATGGTGAAGGGACC-3';

oMODEX-1028: 5'- TTTGCGGCCGCTCATCGCCTCCCTCGGCCTTTCACCAGC-3';

10   oMODEX-1029: 5'- TCCCTTCAGCATGCGAATTCACCCCTGTAG-3';

oMODEX-1030: 5'- ACAGGGGTGAATTCGCATGCTGAAGGGACC-3';

oMODEX-1031h:5'- AGGCCTCCCACGTTCCTCGACTATGGCGAC-3';

oMODEX-1032: 5'- TCGCGACATGCTGAAGGGACCTTTACCAGTG-3';

oMODEX-1033: 5'- TCGCGACATGGTGAAGGGACCTTTACCAGTG-3';

15

Generation of PCR#1 Cassette Containing IgSP-GLP1-IP2:

The IgSP-GLP1-IP2 chimeric cassette was generated by recombinant PCR.

Briefly, oligonucleotides oMODEX-1025 and –1029 were used to generate the first

PCR product A containing the IgSP coding region whereas oligonucleotides

20   oMODEX-1030 and –1031h were used to generate the first PCR product B containing

the GLP1-IP2 coding region. PCR Products A and B were combined for a second

PCR reaction using flanking oligonucleotides oMODEX-1025 and –1031h to generate

the recombinant IgSP-GLP1-IP2 product which was directly cloned into pCR21.-TOPO

vector by using the TOPO Cloning Kit (Invitrogen). The resulting clone was

25   named as p1035 (refer to Figure 2) and the nucleotide sequence of the cloned IgSP-

GLP1-IP2 recombinant PCR product was verified to be correct.

The IgSP-GLP1(G8)-IP2 chimeric cassette was also generated with the same

protocol as described above for IgSP-GLP1-IP2. The oligonucleotides used for the

first PCR products A and B were oMODEX-1025/1026 and –1027/1031h while the

30   oligonucleotides used for the recombinant PCR reaction were oMODEX-1025 and –

1031h. The resulting clone was named as p1041 (refer to Figure 2) and the nucleotide

sequence of the cloned IgSP-GLP1(G8)-IP2 recombinant PCR product was verified to

be correct.

Generation of PCR#2 Cassette Containing GLP1-IP2:

The GLP1-IP2 and GLP1(G8)-IP2 cassettes were generated by PCR using

oligonucleotides oMODEX-1032/1031h and –1033/-1031h, respectively. The PCR

5   products of GLP1-IP2 and GLP1(G8)-IP2 were directly cloned into pCR21.-TOPO

vector by using the TOPO Cloning Kit (Invitrogen) generating p1037 and p1043,

respectively (refer to Figure 2). The nucleotide sequences of the cloned GLP1-IP2

and GLP1(G8)-IP2 PCR products were verified to be correct.

10   **Generation of PCR#3 Cassette Containing IP2:**

The IP2 and IP2(G8) cassettes were generated by PCR using oligonucleotides

oMODEX-1032/1028 and –1033/-1028, respectively. The PCR products of IP2 and

IP2(G8) were directly cloned into pCR21.-TOPO vector by using the TOPO Cloning

Kit (Invitrogen) generating p1039 and p1045, respectively (refer to Figure 2). The

15   nucleotide sequences of the cloned IP2 and IP2(G8) PCR products were verified to

be correct.

Construction of the Multimeric IgSP-GLP1-IP2 Expression cassette:

The general strategy for generating multimeric GLP1-IP2 coding regions is

20   depicted in Figure 1. For the IgSP-GLP1-IP2, the basic building blocks are the

PCR#1, PCR#2, and PCR#3 in plasmids p1035, p1037, and p1039, respectively (refer

to Figure 2). For the IgSP-GLP1(G8)-IP2, the basic building blocks are the PCR#1,

PCR#2, and PCR#3 in plasmids p1041, p1043, and p1045 (refer to Figure 3). These

basic building blocks can be pieced together to generate an unlimited number of

25   multimeric GLP1-IP2 expression cassettes as shown in Figure 1. Table 1 lists the

intermediary multimeric GLP1 constructs for both wild type and G8 versions in

pCR2.1-TOPO.

Construction of pPI-DN-Based Expression Vectors:

30   The final multimeric GLP1 expression cassettes, designated Final 2, Final 3,

Final 4, and Final 5 were subcloned into the expression vector pPI-DN (refer to Figure

3) generating p1062, p1068, p1079, and p1085, respectively. Similarly, the final

multimeric GLP1(G8) expression cassettes of Final 2, Final 3, Final 4, and Final 5
were also subcloned into the expression vector pPI-DN generating p1064, p1070,
p1081, and p1087, respectively (refer to Figure 3). Briefly, the multimeric GLP1
expression cassettes were excised out of the pCR2.1-TOPO-based clones by NheI and

5   NotI digestions and subsequently subcloned into pPI-DN that was also digested with
NheI and NotI. All the resulting clones are in the sense orientation.

Generation of Stable BHK Cell Lines:

All the pPI-DN-based multimeric GLP1 expression vectors were stably

10   transfected into BHK cells by using the Lipofectamine Plus (Gibco Life-Technology,
MD). Briefly, the selection drug G418 was added to the transfected BHK cells 24 hours
after transfection. Stably transfected p1091/BHK ells were grown in DMEM plus
10% FBS and 1 mg/ml of G418 for 2 weeks. One hundred thousand cells of each
stable BHK cell lines were seeded and pulsed with 1 ml of DMEM plus 10% for 24

15   hours. The amount of secreted GLP1 and GLP1(G8) peptides was analyzed by RIA.

Results

As shown in Figure 4, all versions of multimeric GLP1 expression cassettes
can direct high levels of GLP1 secretion in stable BHK cells. The 2-mer, 3-mer, and

20   4-mer wild type GLP1 expression cassettes were shown to secret GLP1 at 40+8,
115+1, and 116+21 ng/million cells/24 hrs, respectively. The 2-mer, 3-mer, and 4-
mer GLP1(G8) expression cassettes were shown to secrete GLP1(G8) at 82+7,
250+17, and 441+130 ng/million cells/24 hrs, respectively.

25   **Example 5** **Encapsulation of GLP-1 secreting cells**

One suitable encapsulation procedure is as follows: The hollow fibers were
fabricated from a polyether sulfone (PES) with an outside diameter of 720 μm and a
wall thickness of a 100 μm (AKZO-Nobel Wüppertal, Germany). These fibers are
described in United States patents 4,976,859 and 4,968,733, herein incorporated by

30   reference. In some studies we use a PES#5 membrane which has a MWCO of about
280 kd. In other studies we use a PES#8 membrane which has a MWCO of about 90
kd.

The devices typically comprise:

1) a semipermeable poly (ether sulfone) hollow fiber membrane fabricated by AKZO Nobel Faser AG;

2) a hub membrane segment;

3) a light cured methacrylate (LCM) resin leading end; and

4) a silicone tether.

The semipermeable PES membrane used typically have the following characteristics:

Internal Diameter	500 ± 30 μm
Wall Thickness	100 ± 15 μm
Force at Break	100 ± 15 cN
Elongation at Break	$44 \pm 10\%$
Hydraulic Permeability	63 ± 8 (ml/min m^2 mmHg)
nMWCO (dextrans)	280 ± 20 kd

The components of the device are commercially available. The LCM glue is available from Ablestik Laboratories (Newark, DE); Luxtrak Adhesives LCM23 and LCM24). The tether material is available from Specialty Silicone Fabricators (Robles, CA). The tether dimensions are 0.79 mm OD x 0.43 mm ID x length 202 mm. The morphology of the device is as follows: The inner surface has a permselective skin. The wall has an open cell foam structure. The outer surface has an open structure, with pores up to 1.5 μm occupying $30 \pm 5\%$ of the outer surface.

Fiber material is first cut into 5 cm long segments and the distal extremity of each segment sealed with a photopolymerized acrylic glue (LCM-25, ICI). Following sterilization with ethylene oxide and outgassing, the fiber segments are loaded with a suspension of transfected cells in an alginate or collagen solution (Zyderm® soluble bovine collagen) via a Hamilton syringe and a 25 gauge needle through an attached injection port. The proximal end of the capsule was sealed with the same acrylic glue. In some studies the collagen matrix was Zyplast™. The volume of the device contemplated in the human studies is approximately 15-18 μl.

A silicone tether (Specialty Silicone Fabrication, Taunton, MA) (ID: 690 μm; OD: 1.25 mm) is placed over the proximal end of the fiber allowing easy manipulation and retrieval of the device.

<u>Example 6</u> **Delivery of GLP-1**

      As shown in Figure 5, diabetic rats, 33 days post implantion with GLP-1 secreting encapsulated cell devices are able to properly glucose regulate virtually

5   indistinguishable from non-diabetic control, compared with untreated diabetic controls.

      In addition, as shown in Figure 6, diabetic rats with GLP-1 secreting device implants showed markedly reduce weight gain compared to diabetic control counterparts.

WE CLAIM:

1. A method of treating type II diabetes mellitus in an individual suffering therefrom comprising the steps of :

    administering to the patient preprandially a therapeutic amount of GLP-1 or a GLP-1 mutein wherein said GLP-1 mutein is a insulinotropic peptide having the formula:

    His-Xaa^1-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Xaa^2-Gly-Gln-Ala-Ala-Xaa^3-Xaa^4-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Xaa^5 wherein

Xaa^1	Gly, ;
Xaa^2	is Glu, Gln, Ala, Thr, Ser, and Gly;
Xaa^3	is Lys, and Arg;
Xaa^4	is Glu, Gln, Ala, Thr, Ser, and Gly; and
Xaa^5	is Gly-OH or is absent;

    wherein the amount of said GLP-1 or GLP-1 mutein is sufficient to mitigate a diabetic event in said patient.

2. The method of claim 1 wherein said therapeutically effective amount of said insulinotropic peptide is delivered 0.5 to 4.0 hours preprandially.

3. The method of claim 1 wherein said administration is via injection.

4. The method of claim 1 wherein said administration is via a pharmaceutically acceptable oral formulation.

5. The method of claim 1 wherein said administration is via implanted cells or tissue.

6. The method of claim 5 wherein said tissue or cells are encapsulated in an encapsulation device.

7. The method of claim 1 wherein the insulinotropic peptide is GLP-1 administered at a dose of about 0.1 - 80 µg/day.

8.   The method of claim 1 wherein the insulinotropic peptide is GLP-1 administered at
     a dose of about 0.5- 2.5 µg/day.

9.   The method of claim 1 wherein the insulinotropic peptide is GLP-1-Gly$_8$
     administered at a dose of about 0.0125 - 2.5 µg/day.

10. The method of claim 1 wherein the insulinotropic peptide is GLP-1-Gly$_8$
     administered at a dose of about 0.05- 2.0 µg/day.

11. A method for treating obesity in a patient suffering from type II diabetes in a patient
     suffering therefrom comprising delivering a continuous source of GLP-1 to a
     patient at a dosage to the patient at a dosage of about 0.1 - 80 µg/day.

12. The method of claim 11 wherein the dosage is between 0.5- 2.5 µg/day.

13. The method of claim 11 wherein the continuous source of GLP-1 is encapsulated
     cells or tissue that secrete the GLP-1 into the patient.

14. A method for treating obesity in a patient suffering from type II diabetes in a patient
     suffering therefrom comprising delivering a continuous source of GLP-1-Gly$_8$ to a
     patient at a dosage to the patient at a dosage of about 0.0125 - 2.5 µg/day.

15. The method of claim 14 wherein the dosage is between 0.05- 2.0 µg/day.
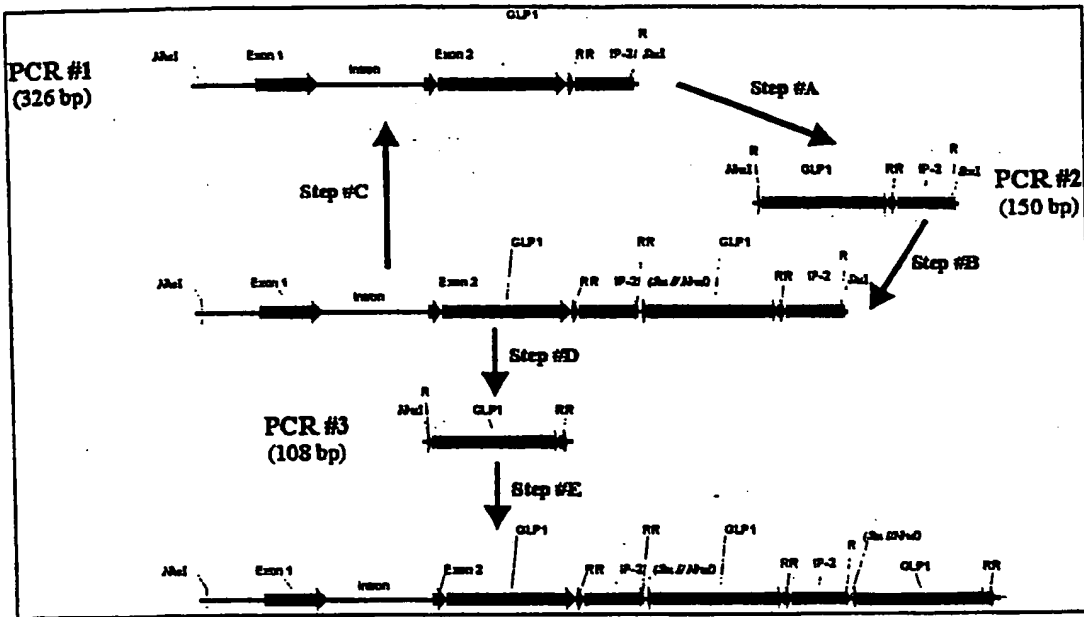
Figures

Figure 1. General Strategy for Generating the Multimeric IgSP-GLP1-IP2 Expression
Cassettes. PCR#1, PCR#2, and PCR#3 represent plasmids containing the basic building
blocks of IgSP-GLP1-IP2, GLP1-IP2, and IP2, respectively, as well as IgSP-GLP1(G8)-IP2,
GLP1(G8)-IP2, and IP2(G8), respectively.
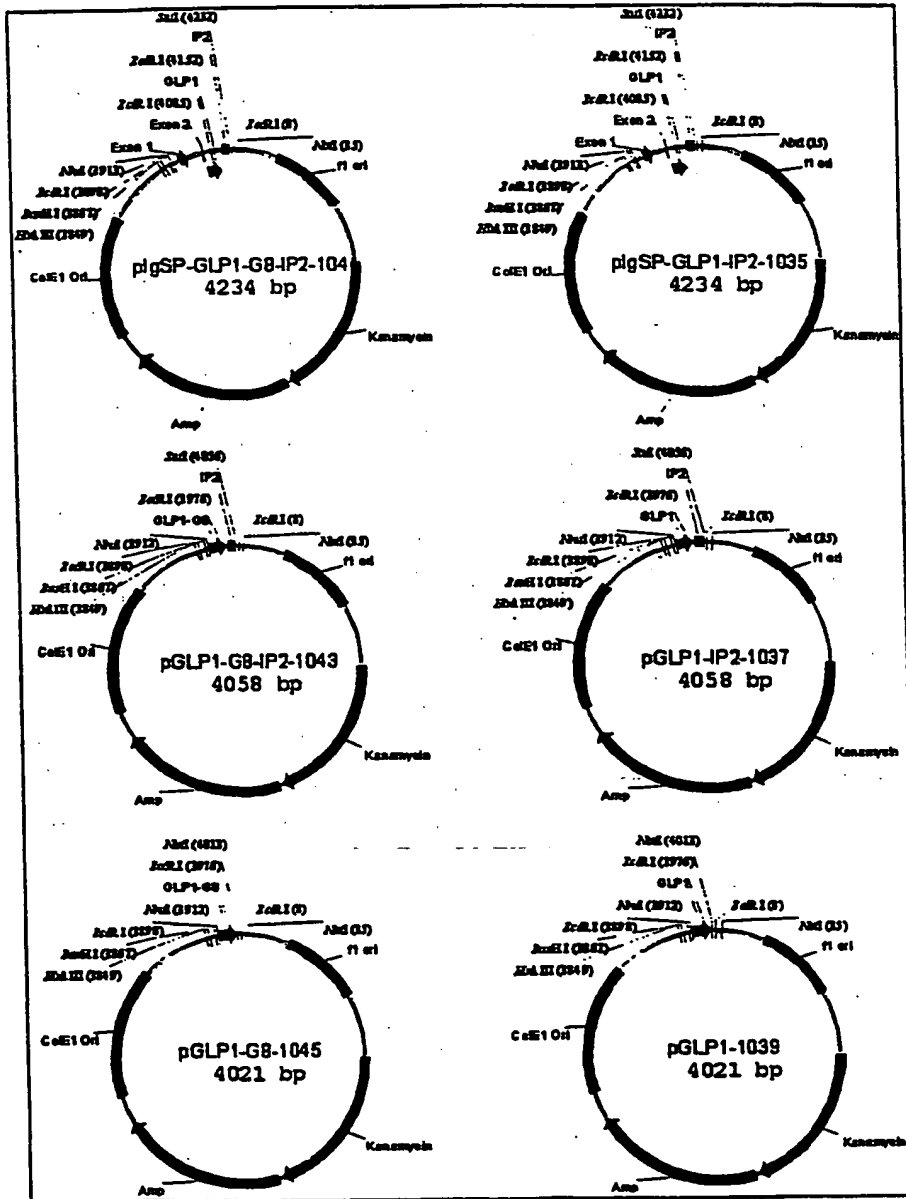
Figure 2: Plasmid Maps of the Starting Plasmids Containing the Building Blocks for the Construction of Multimeric IgSP-GLP1-IP2 and IgSP-GLP1(G8)-IP2 Expression Cassettes. Plasmids p1041, p1043, and p1045 are the building block constructs for IgSP-GLP1-IP2 whereas plasmids p1035, p1037, and p1039 are for IgSP-GLP1(G8)-IP2. All the cloned PCR products were verified to be correct by nucleotide sequence determination.

GLP-1		GLP-1-G8			
pCR2.1	pPI-DN	pCR2.1	pPI-DN		
p1035 √		p1041 √		▮ ▮	Intermediary 1
p1061 √	p1062 √+	p1063 √	p1064 √+	▮ ▮	Final 2
p1065 √		p1066 √		▮ ▮	Intermediary 2
p1067 √	p1068 √+	p1069 √	p1070 √+	▮ ▮	Final 3
p1071 √		p1072 √		▮ ▮	Intermediary 3
p1078 √	p1079 √+	p1080 √	p1081 √+	▮ ▮	Final 4
p1082 √		p1083 √		▮ ▮	Intermediary 4
p1084 √	p1085 √	p1086 √	p1087 √	▮ ▮	Final 5

Figure 3. A List of Multimeric GLP1 Expression Cassettes in pCR2.1-TOPO and pPI-DN vectors. GLP-1 and GLP-1-G8 designate the wild type and Gly-8 mutant GLP1 multimeric cassettes. pCR2.1 and pPI-DN represent clones in pCR2.1-TOPO cloning vector and pPI-DN expression vector, respectively. The check mark represents that the construct was already made whereas the plus sign indicates the expression of GLP1 peptides to be positive from BHK cells as determined by RIA.
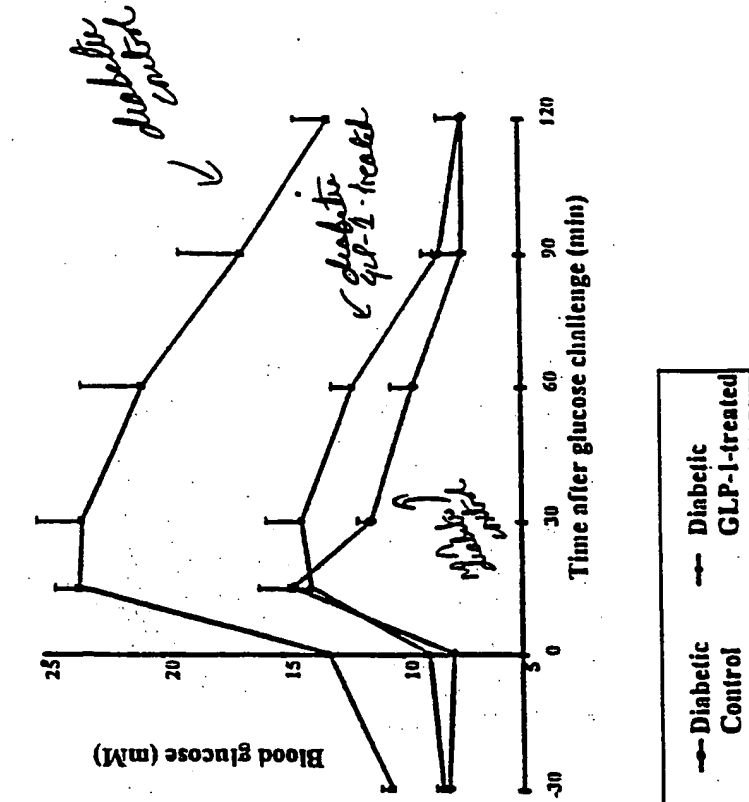
GLP1 Secretion from BHK Cells
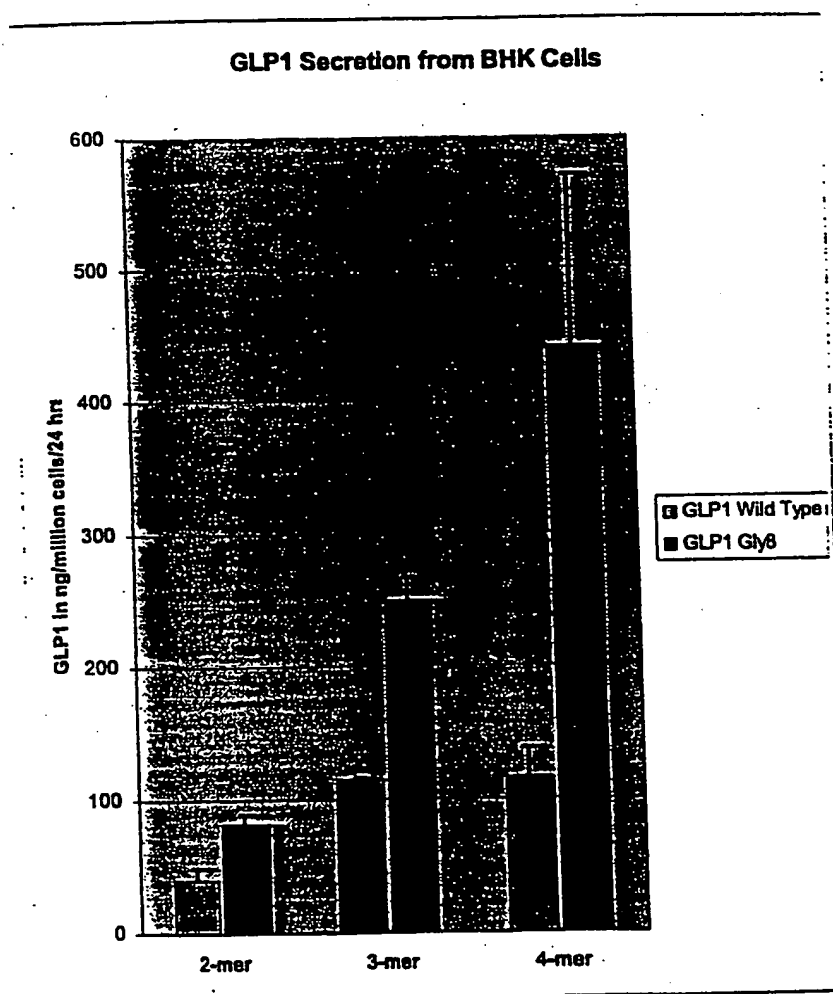
Figure 4. GLP1 Secretion from BHK Cells. Multimeric GLP1 constructs with 2, 3, and 4 copies of GLP1 (wild type) and GLP1(G8) are designated as 2-mer, 3-mer, and 4-mer, respectively. The GLP1 output was determined by RIA and expressed in ng per million cells per 24 hours.
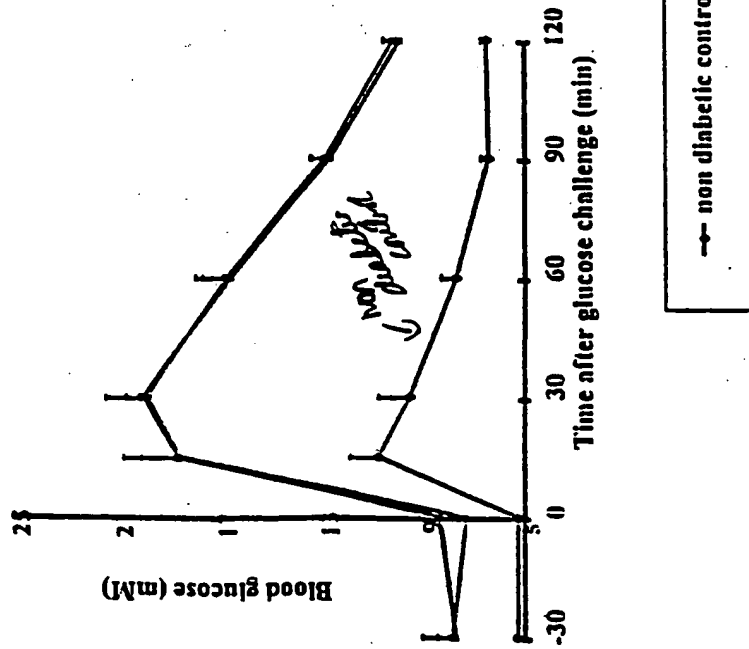
IPGTT

33 day post-implantation

diabetic control

diabetic GLP-1-treated

non diabetic control

Blood glucose (mM)

Time after glucose challenge (min)

-30   0   30   60   90   120

Preimplantation

non diabetic control

Blood glucose (mM)

Time after glucose challenge (min)

-30   0   30   60   90   120

| non diabetic control | Diabetic Control | Diabetic GLP-1-treated |

FIG. 5

FIG. 6